PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference 686338C:MOB	**FOR FURTHER ACTION**	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416).
International Application No. **PCT/AU2003/001688**	International Filing Date *(day/month/year)* 18 December 2003	Priority Date *(day/month/year)* 18 December 2002
International Patent Classification (IPC) or national classification and IPC **Int. Cl.** [7] A61K 39/12, 39/125, A61P 35/00		
Applicant THE UNIVERSITY OF NEWCASTLE RESEARCH ASSOCIATES LIMITED et al		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 5 sheets, including this cover sheet.

   [X] This report is also accompanied by ANNEXES, i.e., sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of 8 sheet(s).

3. This report contains indications relating to the following items:

I	[X]	Basis of the report
II	[ ]	Priority
III	[ ]	Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
IV	[ ]	Lack of unity of invention
V	[X]	Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
VI	[ ]	Certain documents cited
VII	[ ]	Certain defects in the international application
VIII	[X]	Certain observations on the international application

Date of submission of the demand 12 July 2004	Date of completion of the report 22 March 2005
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Form PCT/IPEA/409 (Cover sheet) (July 1998)

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No.
PCT/AU2003/001688

I. Basis of the report

1.* With regard to the elements of the international application:*

- [ ] the international application as originally filed.
- [X] the description, pages **1-13 ,16-36** as originally filed,
  pages , filed with the demand,
  pages **14,15** received on **4 March 2005** with the letter of **1 March 2005**
- [X] the claims, pages , as originally filed,
  pages , as amended (together with any statement) under Article 19,
  pages , filed with the demand,
  pages **37-42,** received on **4 March 2005** with the letter of **1 March 2005**
- [X] the drawings, pages **1/19-19/19,** as originally filed,
  pages , filed with the demand,
  pages , received on with the letter of
- [ ] the sequence listing part of the description:
  pages , as originally filed
  pages , filed with the demand
  pages , received on with the letter of

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.
These elements were available or furnished to this Authority in the following language which is:
- [ ] the language of a translation furnished for the purposes of international search (under Rule 23.1(b)).
- [ ] the language of publication of the international application (under Rule 48.3(b)).
- [ ] the language of the translation furnished for the purposes of international preliminary examination (under Rules 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:
- [ ] contained in the international application in written form.
- [ ] filed together with the international application in computer readable form.
- [ ] furnished subsequently to this Authority in written form.
- [ ] furnished subsequently to this Authority in computer readable form.
- [ ] The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.
- [ ] The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished

4. [ ] The amendments have resulted in the cancellation of:
   - [ ] the description, pages
   - [ ] the claims, Nos.
   - [ ] the drawings, sheets/fig.

5. [ ] This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed, as indicated in the Supplemental Box (Rule 70.2(c)).**

* *Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rules 70.16 and 70.17).*

** *Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report*

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V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Claims	1-63	YES
	Claims		NO
Inventive step (IS)	Claims	1-63	YES
	Claims		NO
Industrial applicability (IA)	Claims	1-63	YES
	Claims		NO

2. Citations and explanations (Rule 70.7)

The following documents identified in the International Search Report have been considered for the purposes of this report:

D1: Ferdat, AK et al
D2: WO 2001/037866
D3: Taguchi, F

Novelty (N): Claims 1-63

The invention is directed to the treatment of abnormal cells for example, cancerous cells, with an effective amount of an oncolytic virus selected from echoviruses and modified forms or combinations thereof, that recognises $\alpha_2\beta_1$ for the infectivity of the cells such that at least some of the cells are killed by the virus. The invention also encompasses the screening of abnormal cells for susceptibility to said virus and pharmaceutical compositions comprising the virus as an inoculant, together with a pharmaceutically acceptable carrier and the use thereof.

D1 teaches that the local administration of human echo-7 virus exerts an inhibitory effect on the growth of MX-17 tumour in BALB/c mice without signs of oncolysis. The document does not disclose nor suggest that at least some of the cells are killed by the virus.

D2 discloses a method of treating a malignancy for example, melanoma cells, with a virus that recognises a cell adhesion molecule and a complement regulatory protein, where the virus preferably is derived from the *Picornaviridae* family, e.g. coxsackievirus and echovirus. Specifically, the document discloses the use of echovirus type 7 (EV7) for infection of melanoma cells. However, the document does not disclose nor suggest that the virus recognise $\alpha_2\beta_1$ for the infectivity of the cells and eventual killing of the cells. It has been demonstrated by the applicant that EV7 infection involves the interaction of EV7 with the complementary regulatory protein, decay accelerating factor (DAF) not $\alpha_2\beta_1$.

D3 teaches the mass culture of viruses including echovirus that are used as inoculants for vaccines or antigens for diagnosis. However, it does not teach the killing of the abnormal cells. The document exemplifies only the preparation of smallpox vaccine liquor. Thus, it does not disclose all the essential features of the present invention.

Therefore the subject matter of these claims is new and meets the requirements of Article 33(2) PCT with regard to novelty.

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Supplemental Box
(To be used when the space in any of the preceding boxes is not sufficient)

Continuation of

Inventive Step (IS): Claims 1-63

Claims 1-63 meet the criteria set out in PCT Article 33(3) with regard to the requirement of Inventive Step because the prior art does not obviously suggest to a person skilled in the art to treat abnormal cells with echoviruses and modified forms that recognises $\alpha_2\beta_1$ for the infectivity of the cells and such that at least some of the cells are killed by the virus. With respect to D3 the applicant has distinguished the teachings of said document on the basis of the physicochemical and biological differences between the small pox virus and echovirus. On the basis of this, it is considered that the skilled addressee would not be directly led to prepare a pharmaceutical composition of echovirus of the present invention.

Industrial Applicability (IA) Claims 1-63

The invention defined in the claims is considered to meet the requirements of Industrial Applicability under Article 33(4) of the PCT because it can be made by, or used in, industry.

VIII. Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:

Claims 58-62 do not fully describe the invention. The claims are directed to an applicator comprising a region that is impregnated with an inoculant consisting of echovirus, modified forms or a combination thereof, that recognises $\alpha_2\beta_1$ for the infectivity of the cells. However, it appears from the applicant's response that an essential feature of the invention is that the inoculant kills at least some of the cells. This feature is not defined in the claims.

Claim 58 is not clear in that there appears to be a typographical error in the phrase "impregnated with the inoculant **mammal**".

The biopsies may be taken from different sites of a single individual or from a number of individuals.

A virus used in a method as described herein will desirably cause few or only minor clinical symptoms in the recipient. Such viruses are readily obtainable from commercial sources well known to the skilled addressee and can be screened for their effectiveness in the instant methods in the manner described above. Desirably, the virus will normally be an echovirus selected from the group consisting of Echovirus EV1 and Echovirus EV8. Each of these viruses recognise $\alpha_2\beta_1$ for cell infectivity. EV1 has for instance been associated with mild upper respiratory illnesses and also pleurodynia (Fields B. N. et al, 2000; McCracken A. W. et al, 1969).

The expression of $\alpha_2\beta_1$ is believed to be upregulated on ovarian carcinomas due to the prevalent collagen I matrix it encounters in the mesothelial. Numerous malignant melanomas have also been shown to express upregulated levels of $\alpha_2\beta_1$ (Kramer R. H. and Marks N, 1989; Ramos D. M. etal, 1990). EV1 and collagen attach to $\alpha_2\beta_1$ using different residues in domain I of the $\alpha_2\beta_1$ subunit (Bergelson J.H. 1993). The integrin $\alpha_2\beta_1$ cannot simultaneously accommodate EV1 and collagen. However, the virus binds $\alpha_2\beta_1$ with a 10-fold increase in affinity compared to collagen I (Xing L, 2002).

For the purpose of screening a given virus to ascertain whether it is capable of infecting and causing the death of malignant cells, malignant cell lines may be used rather than primary malignant cells isolated from a biopsy.

The selected virus will preferably be injected directly into a number of sites on a malignant tumor in order to maximise the area for potential infection of the tumor by the virus. Rather than intact virus, viral or other plasmids or expression vectors incorporating nucleic acid for generation of the virus may be injected into the tumor for uptake by tumor cells and generation of intact virus within the cells for effecting the treatment. Suitable expression vectors include plasmids capable of expression of a DNA (eg genomic DNA or cDNA) insert encoding viral proteins necessary for generation of the virus. An expression vector will typically include transcriptional regulatory control sequences to which the inserted nucleic acid is operably linked. By "operably linked" is meant the nucleic acid insert is linked to the transcriptional regulatory control sequences for permitting transcription of the inserted sequence(s) without a shift in the reading frame of the insert. Such transcriptional regulatory control sequences include promotors

for facilitating binding of RNA polymerase to initiate transcription, and expression control elements for enabling binding of ribosomes to transcribed mRNA.

More particularly, the term "regulatory control sequence" as used herein is to be taken to encompass any DNA that is involved in driving transcription and controlling (ie regulating) the level of transcription of a given DNA sequence. For example, a 5' regulatory control sequence is a DNA sequence located upstream of a coding sequence and which may comprise the promotor and the 5'untranslated leader sequence. A 3' regulatory control sequence is a DNA sequence located downstream of the coding sequence(s), which may comprise suitable transcription termination (and/or) regulation signals, including one or more polyadenylation signals. As used herein, the term "promotor" encompasses any DNA sequence which is recognised and bound (directly or indirectly) by a DNA-dependant RNA polymerase during initiation of transcription. A promotor includes the transcription initiation site, and binding sites for transcription initiation factors and RNA polymerase, and can comprise various other sites or sequences (eg enhancers), to which gene expression regulatory proteins may bind.

Numerous expression vectors suitable for transfection of mammalian cells are known in the art. Expression vectors suitable for transfection of mammalian cells include pSV2neo, pEF-PGk. puro, pTk2 and non-replicating adenoviral shuttle vectors incorporating the polyadenylation site and elongation factor 1-x promotor and pAdEasy based expression vectors most preferably incorporating a cytomegalovirus (CMV) promotor (eg see He et al, 1998). The plasmid pEFBOS which employs the polypeptide elongation factor- alpha 2 as the promotor may also be utilised.

cDNA encoding the viral proteins necessary for generation of the virus may be prepared by reverse transcribing the viral RNA genome or fragments thereof and incorporated into a suitable vector utilising recombinant techniques well known in the art as described in for example Sambrook et al (1989), Molecular Cloning: A Laboratory Manual, Second Ed., Cold Spring Harbour Laboratory Press, New York, and Ausubel et al. , (1994), Current Protocols in Molecular Biology, USA, Vol. 1 and 2.

Rather than cDNA, cells may be transfected with viral RNA extracted from purified virions or for instance RNA transcripts may be generated invitro from xDNA templates utilising bacteriophage T7 RNA polymerase as described in Ansardi .D C., et al, 2001. Similarly, a single plasmid or RNA molecule may be administered for expression of viral proteins and generation of virus, or a plurality of plasmids or RNA molecules encoding

Claims:

1. A method for treatment of abnormal cells in a mammal, the method comprising treating the mammal with an effective amount of virus selected from echoviruses, and modified forms and combinations thereof, which recognise $\alpha_2\beta_1$ for infectivity of the cells such that at least some of the cells are killed by the virus.

2. A method according to claim 1 comprising subjecting the mammal to a number of treatments with the virus, and the virus in each of the treatments is the same or different.

3. A method according to claim 1 wherein the virus comprises an echovirus serotype or modified form thereof.

4. A method according to claim 3 wherein the virus is selected from the group consisting of EV1 and EV8.

5. A method according to claim 3 wherein the virus is a modified echovirus.

6. A method according to claim 5 wherein the virus has been modified to enhance ability of the virus to infect the abnormal cells.

7. A method according to claim 5 or 6 wherein the modified echovirus is a modified form of an echovirus selected from a group consisting of EV1 and EV8.

8. A method according to any one of claims 1 to 7 wherein the virus is administered to the mammal in combination with a further virus which infects the abnormal cells.

9. A method according to claim 8 wherein the abnormal cells express ICAM-1 and the further virus recognises ICAM-1 for infectivity of the abnormal cells.

10. A method according to claim 9 wherein the further virus is a Coxsackievirus or modified form thereof.

11. A method according to claim 10 wherein the Coxsackievirus is a Coxsackievirus serotype selected from A13, A15, A18 and A21.

12. A method according to any one of claims 1 to 11 wherein the abnormal cells are cancer cells.

13. A method according to claim 12 wherein the cancer cells are cells of a cancer selected from a group consisting of ovarian cancer, melanoma, prostate cancer, breast cancer, pancreatic cancer, colon cancer and colorectal cancer, or have spread from ovarian cancer, melanoma, prostate cancer, breast cancer, pancreatic cancer, colon cancer or colorectal cancer.

14. A method according to any one of claims 1 to 13 wherein the abnormal cells have up-regulated expression $\alpha_2\beta_1$.

15. A method according to any one of claims 1 to 14 wherein the virus is administered topically, systemically or intratumorally to the mammal.

16. A method of screening a sample of abnormal cells from a mammal for susceptibility to virus induced cell death to evaluate administering virus to the mammal for treatment of the abnormal cells, the method comprising:

(a) providing the sample of the abnormal cells;

(b) treating the cells with the virus for a period of time sufficient to allow infection of the cells by the virus; and

(c) determining whether the virus has infected and caused death of at least some of the abnormal cells;

wherein the virus is selected from echoviruses, and modified forms and combinations thereof, which recognise $\alpha_2\beta_1$ for infectivity of the abnormal cells.

17. A method according to claim 16 wherein the virus comprises an echovirus serotype or a modified form thereof.

18. A method according to claim 16 wherein the virus is selected from a group consisting of EV1 and EV8.

19. A method according to claim 17 wherein the virus is a modified echovirus.

20. A method according to claim 19 wherein the virus has been modified to enhance ability of the virus to infect the abnormal cells.

21. A method according to claim 19 or 20 wherein the modified echovirus is a modified form of an echovirus selected from a group consisting of EV1 and EV8.

22. A method according to any one of claims 16 to 21 further comprising comparing ability of the virus to infect and cause death of the cells with a different virus subjected to steps (b) and (c) utilising another sample of the cells and which recognises $\alpha_2\beta_1$ for infectivity of the cells.

23. A method according to claim 22 wherein the different virus is a different echovirus or modified form thereof.

24. A method according to any one of claims 16 to 23 wherein the cells are cancer cells.

25. A method according to claim 24 wherein the cancer cells are cells of a cancer selected from a group consisting of ovarian cancer, melanoma, prostate cancer, breast cancer, pancreatic cancer, colon cancer and colorectal cancer, or have spread from

ovarian cancer, melanoma, prostate cancer, breast cancer, pancreatic cancer, colon cancer or colorectal cancer.

26. A method of screening a virus for ability to infect and cause death of abnormal cells from a mammal to evaluate administering the virus to the mammal for treatment of the abnormal cells, the method comprising:

(a) selecting the virus;

(b) treating a sample of the abnormal cells from the mammal with the virus for a period of time sufficient to allow infection of the cells by the virus; and

(c) determining whether the virus has infected and caused death of at least some of the abnormal cells;

wherein the virus is selected from echoviruses and modified forms thereof, which recognise $\alpha_2\beta_1$ for infectivity of the abnormal cells.

27. A method according to claim 26 wherein the virus comprises an echovirus serotype or a modified form thereof.

28. A method according to claim 26 wherein the virus is selected from a group consisting of EV1 and EV8.

29. A method according to claim 27 wherein the virus is a modified echovirus.

30. A method according to claim 29 wherein the virus has been modified to enhance the ability of the virus to infect the abnormal cells.

31. A method according to claim 29 or 30 wherein the modified echovirus is a modified form of an echovirus selected from a group consisting of EV1 and EV8.

32. A method according to any one of claims 26 to 31 further comprising comparing ability of the virus to infect and cause death of the cells with a different virus subjected to steps (b) and(c) utilising another sample of the cells and which recognises $\alpha_2\beta_1$ for infectivity of the cells.

33. A method according to claim 32 wherein the different virus is a different echovirus or modified form thereof.

34. A method according to any one of claims 26 to 33 wherein the abnormal cells are cancer cells.

35. A method according to claim 34 wherein the cancer cells are cells of a cancer selected from a group consisting of ovarian cancer, melanoma, prostate cancer, breast cancer, pancreatic cancer, colon cancer and colorectal cancer, or have spread from ovarian cancer, melanoma, prostate cancer, breast cancer, pancreatic cancer, colon cancer or colorectal cancer.

36. A method for inducing an immune response in a mammal against abnormal cells expressing $\alpha_2\beta_1$, the method comprising infecting abnormal cells in the mammal with virus selected from echoviruses, and modified forms and combinations thereof, whereby lysis of at least some of cells is caused.

37. A method according to claim 36 wherein the virus comprises an echovirus serotype of modified form thereof.

38. A method according to claim 37 wherein the virus is selected from the group consisting of EV1 and EV8.

39. A method according to claim 37 wherein the virus is a modified echovirus.

40. A method according to claim 39 wherein the virus has been modified to enhance ability of the virus to infect the abnormal cells.

41. A method according to claim 39 or 30 wherein the modified echovirus is a modified form of an echovirus selected from a group consisting of EV1 and EV8.

42. A method according to any one of claims 36 to 41 wherein the abnormal cells have up-regulated expression $\alpha_2\beta_1$.

43. A method according to any one of claims 36 to 42 wherein the virus is administered to the mammal in combination with a further virus which infects the abnormal cells.

44. A method according to claim 43 wherein the abnormal cells express ICAM-1 and the further virus recognises ICAM-1 for infectivity of the abnormal cells.

45. A method according to claim 44 wherein the further virus is a Coxsackievirus or modified form thereof.

46. A method according to claim 45 wherein the Coxsackievirus is a Coxsackievirus serotype selected from A13, A15, A18 and A21.

47. A method according to any one of claims 36 to 46 wherein the abnormal cells are cancer cells.

48. A method according to claim 47 wherein the cancer cells are cells of a cancer selected from a group consisting of ovarian cancer, melanoma, prostate cancer, breast cancer, pancreatic cancer, colon cancer and colorectal cancer, or have spread from ovarian cancer, melanoma, prostate cancer, breast cancer, pancreatic cancer, colon cancer or colorectal cancer.

49. A method according to any one of claims 36 to 48 wherein the virus is administered topically, systemically or intratumorally to the mammal.

50. A pharmaceutical composition for treating abnormal cells in a mammal, comprising an inoculant for generating virus to treat the cells such that at least some of

the cells are killed by the virus together with a pharmaceutically acceptable carrier, wherein the virus is selected from echoviruses, and modified forms and combinations thereof, which recognise $\alpha_2\beta_1$ for infectivity of the cells.

51. A pharmaceutical composition according to claim 50 wherein the virus comprises an echovirus serotype or modified form thereof.

52. A pharmaceutical composition according to claim 51 wherein the virus is selected from the group consisting of EV1 and EV8.

53. A pharmaceutical composition according to claim 49 wherein the virus is a modified echovirus.

54. A pharmaceutical composition according to claim 51 wherein the virus has been modified to enhance ability of the virus to infect the abnormal cells.

55. A pharmaceutical composition according to claim 53 or 54 wherein the modified echovirus is a modified form of an echovirus selected from a group consisting of EV1 and EV8.

56. A pharmaceutical composition according to any one of claims 50 to 55 wherein the abnormal cells are cancer cells.

57. A pharmaceutical composition according to any one of claims 50 to 56 wherein the pharmaceutical composition is for topical administration or injection.

58. An applicator for applying an inoculant to a mammal for generating virus to treat abnormal cells in the mammal, wherein the applicator comprises a region impregnated with the inoculant mammal such that the inoculant is in contact with the mammal, and the virus is selected from echoviruses, and modified forms and combinations thereof, which recognise $\alpha_2\beta_1$ for infectivity of the cells..

59. An applicator according to claim 58 wherein the region impregnated with the virus comprises padding or wadding for being held in contact with the mammal.

60. An applicator according to claim 58 or 59 wherein the abnormal cells are abnormal skin cells and the applicator further comprises one or more adhesive surfaces for adhering to skin of the mammal.

61. An applicator according to any one of claims 58 to 60 in the form of a patch or sticking plaster.

62. Use of an inoculant for generating virus in the manufacture of medicament for inducing an immune response against abnormal cells in a mammal, where the virus is selected from echovirus, and modified forms and combinations thereof, which recognise $\alpha_2\beta_1$, for infectivity of the abnormal cells.

63. Use of an inoculant for generating virus in the manufacture of medicament for inducing an immune response against abnormal cells in a mammal, where the virus is selected from echovirus, and modified forms and combinations thereof, which recognise $\alpha_2\beta_1$, for infectivity of the abnormal cells and kill the cells.